Chemosphere 182 (2017) 720–729

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Antimicrobial agents, triclosan, chloroxylenol, methylisothiazolinone and borax, used in cleaning had genotoxic and histopathologic effects on rainbow trout

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HIGHLIGHTS

- MIT, TRC and PCMX were genotoxic.
- Genes were significantly regulated by TRC, PCMX, MIT and BRX.
- TRC, PCMX, MIT and BRX caused histopathological effects.
- Chronic toxic effect of TRC, PCMX, MIT, and BRX is high in rainbow trout.

ARTICLE INFO

Article history:
Received 8 March 2017
Received in revised form
9 May 2017
Accepted 15 May 2017
Available online 15 May 2017

Handling Editor: Jim Lazorchak

Keywords:
Comet assay
Genotoxicity
Gene expression
Histopathology

ABSTRACT

Triclosan (TRC), chloroxylenol (PCMX) and methylisothiazolinone (MIT) have been commonly used as an antimicrobial in soaps while borax (BRX) is used in household cleaning. After using these chemicals, they are washed down drains and getting into the aquatic ecosystem in which they may affect aquatic living organisms. In the present study, the chronic effects of TRC, PCMX, MIT and BRX on genotoxicity, gene expression and histopathology of rainbow trout (*Oncorhynchus mykiss*) were evaluated for 40 days under semi static condition. The comet assay results indicated that MIT, TRC and PCMX caused significant DNA damage to erythrocytes of the fish. Transcription of *SOD*, *GPX*1, *GPX*2, *GST*A, *HSP90*BB, *HSP90*BA, *CAT*, and *HSC70*A genes were significantly regulated as a result of TRC, PCMX, MIT, and BRX exposure except PCMX exposed *GSTA* gene. Histological lesions were detected in gills, spleen liver, and trunk kidney of the fish. Lamellar fusion, hyperplasia and epithelial necrosis in gills, melanomacrophage centers and splenic necrosis in spleen, pyknotic nucleus, fat vacuoles, necrotic hepatocytes in liver, cloudy swelling in the tubules, renal tubule epithelial cells degeneration, glomerular capillaries dilation and glomerulus degeneration in kidney, were observed. Our study demonstrates the chronic toxic effect of TRC, PCMX, MIT, and BRX is high in rainbow trout. Therefore, we should be more careful when using these chemicals for cleaning in order to protect aquatic environment.

1. Introduction

Antibacterial chemicals also known as antimicrobial pesticides are produced for disinfection, sanitization, decreasing or mitigation of growth or protection of inert objects caused by microorganisms. Nowadays, different kinds of active ingredients have been used in antimicrobial products and they are marketed in many types of formulations including: liquids, concentrated powders, sprays and gases (EPA, 2016). Triclosan, borax and chloroxylenol are commonly used for antibacterial and deodorant effect in consumer products. These chemicals used in hand soaps have been washed down drains and getting into the water system and affecting aquatic environment. These chemicals resistant to degradation and it has been found in streams and wastewater all around the world (Bedoux et al., 2012; Carey and McNamara, 2015; Saleh et al., 2011). There are also some concerns that they have potential to spread antibiotic resistance (Carey and McNamara, 2015; Saleh et al., 2011).

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http://dx.doi.org/10.1016/j.chemosphere.2017.05.093
0045-6535/

The majority of liquid hand soaps and bars use triclosan (TRC) as an active ingredient (Perencevich et al., 2001). Triclosan is a phenoxyphenol antimicrobial, it also has some antiviral and antifungal activity (Bhargava and Leonard, 1996). Recently, it has been placing the chemical all sorts of home items such as in wipes, hand gels, cutting boards and mattress pads to eradicate any bacteria. Increased household sewage discharges can impact water quality in the near shore, an area that is essential for the survival of healthy aquatic organisms (Halden, 2014). TRC is determined in water, wastewater, soil, and sediments (Bedoux et al., 2012; Mavri et al., 2012). This chemical is lipophilic, persistent environmental pollutant thus poses a high risk of bioaccumulation. It is toxic to living organisms in aquatic environments, particularly immediately downstream of effluents from household wastewaters (Brausch and Rand, 2011). Triclosan was detected in many fish species and surface water (Benotti et al., 2009; Dougherty et al., 2010). It has been demonstrated laboratory evidence of triclosan resistance with antibiotics among multiple species of bacteria (Aiello et al., 2004, 2005). However, there are no adequate studies about whether occurrence of antibiotic resistance in environment is associated with the growing use of soaps containing triclosan.

Boric acid (BRX) is a naturally occurring chemicals and found in low concentrations in the environment (EPA, 1998). It is a crucial nutrient for many living organisms although it has become an environmental contaminant due to its widespread use (Topal et al., 2016). Borax has been used in many detergents, cosmetics, and enamel glazes. The aquatic organisms like fish are very sensitive to borax exposure (Schoderboeck et al., 2011). It is toxic to aquatic organisms and toxic concentration varies over a wide range depending on the species and the test conditions (Loewengart, 2001; Schoderboeck et al., 2011). Toxicity of borax was manifested by generalized focal seizure disorders, irritability, and gastrointestinal disturbances. The most common findings were edema and congestion of the brain and meninges. Other common findings included liver enlargement, vascular congestion, fatty changes, swelling, and granular degeneration (Linden et al., 1986; Litovitz et al., 1988; Penland, 1994).

Chloroxylenol (PCMX) and methylisothiazolinone (MIT) are other antimicrobial chemicals using in different products. PCMX is in use as an ingredient to control microorganisms, algae and fungi in paints, emulsions, washing tanks and adhesives. It is also commonly used in antibacterial soaps, wound-cleansing applications and household antiseptics (Bruch, 1986). Toxic effects of PCMX are ranging from low to high, depending on the species and test conditions. While freshwater fish are very susceptible to PCMX, aquatic invertebrates are moderately vulnerable (EPA, 1994). Chloroxylenol is a skin and eye irritant. It can changes blood cellular composition after repeating oral and dermal exposure (Guess and Bruch, 1986). The toxicological data for chloroxylenol lacks of genotoxicity and carcinogenicity (Yost et al., 2016). MIT is a widely used industrial and household biocide and preservative in personal care and cosmetics products such as hand and body lotions, moisturizers, sun tanning lotions and some rinse-off products. In water systems MIT is commonly used antimicrobial to control fungi, slime-forming bacteria and algae. MIT is allergenic and cytotoxic chemical. Chronic exposure to MIT and related compounds may damage to the emerging nervous system (Du et al., 2002). It may cause DNA damage, and affect the enzyme activity as well. Some of industrial products including MIT has been found in estuarine and coastal environments (Voulvoulis et al., 2002).

Because of the widespread use of antimicrobial chemicals, the cellular and molecular effects of these pollutants on fish need to be evaluated. Despite many studies focused on the effects of these chemicals on human is sufficient (Levy, 2002; Pirard et al., 2012), the effects on aquatic ecosystems are not fully illuminated. Although the antimicrobial properties of the disinfectants against some pathogenic microorganisms, ecological risk of this chemical when mixed to receiving water should be thoroughly examined. The objectives of this study were to investigate (1) the chronic toxic effects, (2) histopathological effects (on liver, kidney and gill tissue), (3) blood DNA damage and (4) gene expression effects of TRC, PCMX, MIT, and BRX on rainbow trout (*Oncorhynchus mykiss*).

2. Materials and methods

2.1. Test compounds

Triclosan ($C_{12}H_7Cl_3O_2$, Sigma-Aldrich), chloroxylenol (4-Chloro-3,5-dimethylphenol, Merck Millipore), methyloisothiazolinone (2-Methyl-4-isothiazolin-3-one, Sigma-Aldrich) and borax ($Na_2B_4O_7$, Sigma-Aldrich) were commercially obtained and selected as antibacterial agents for chronic toxicity tests.

Stock solutions of TRC and PCMX were prepared by diluting with ethanol (Merck, ACS reagent, absolute) while MIT and BRX were diluted with distilled water to obtain desired concentrations. During the experiment, daily prepared fresh stock solutions were used.

2.2. Fish

All experiments described in this study were approved by the Institutional Animal Care and Use Committee at Karadeniz Technical University. Rainbow trout (17.77 ± 3.92; 12.04 ± 0.90 cm; Mean ± SD), obtained from Karadeniz Technical University, Faculty of Marine Sciences, were acclimated to laboratory conditions in recirculating systems (200 L) prior to experiments for 30 days. During acclimation and chronic toxicity tests, fish were held under a photoperiod (12 h of light and 12 h of darkness) and fed with commercial trout pellets (4% BW) daily.

During chronic exposure to the antimicrobial chemicals, water characteristics such as temperature, pH, dissolved oxygen, nitrite and ammonia (Boyd and Tucker, 1992) were measured daily in each treatment (Table 1).

2.3. Experimental design

Prior to this study, 96 h LC_{50} concentration for TRC, PCMX, MIT and BRX were found 0.05 mg L^{-1}, 0.043 mg L^{-1}, 0.072 mg L^{-1} and 1 g L^{-1}, respectively (unpublished data). One-hundredth of LC_{50} concentrations of antimicrobials were selected for chronic exposure. Thus, 0.48 ± 0.2 μg L^{-1} of TRC, 4.2 ± 0.9 μg L^{-1} of PCMX, 6.8 ± 1.1 μg L^{-1} of MIT and 8.9 ± 1.8 mg L^{-1} of BRX actual concentrations were used for chronic exposure. MIT and BRX were dissolved in 50 mL distilled water while TRC and PCMX were dissolved in 50 mL ethanol then added test water. Fish were stocked into 3 × 40-l aquaria at a rate of 20 fish/aquarium. Fish were subjected to chronic exposure of TRC, PCMX, MIT and BRX for 40 days after acclimation period. While one of two control groups have only the test water, the other control group was created for TRC and PCMX experiments and prepared with the same amount of ethanol that added to prepare chemical solutions in test groups. During the chronic exposure, fifty-six percent of test solutions and water of control groups were renewed daily (Boran et al., 2010). Fish were sacrificed at 40th day for experimental analysis.

At the beginning of the experiment and then every two days, water samples were taken to determine the actual concentrations of the chemicals in water. HPLC was used for determination of the concentrations of TRC (Zhao et al., 2011), PCMX (Gatti et al., 1997), MIT (Baranowska and Wojciechowska, 2013) and BRX (EPA, 2013) in water.